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LARVICIDAL AND PUPICIDAL EFFECT OF *SPILANTHES ACMELLA* AND *ANDROGRAPHIS PANICULATA* ON THE MOSQUITO *AEDES AEGYPTI*

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ABSTRACT

Keywords:

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Mosquitoes were recognized as a health and nuisance problem only in the last century. They are carriers of number of vector born diseases, such as chikungunya, dengue fever, malaria, filariasis, yellow fever, etc. Although vector control programs have been established for a long time, the main method for control of vectors is the use of chemical insecticides. The conventional chemical pesticides have resulted in the development of resistance, undesirable effects on non-target organism and fostered environmental and human health concerns. An alternative approach for mosquito control is the use of natural products of plant origin. Phytochemicals have proven that they are potential mosquito control agent and also alternative to synthetic insecticides. Hence an attempt has been made to monitor the effect of two-plant extracts (*Spilanthus acmella* and *Andrographis paniculata*) on different larval instar and pupae of mosquito vector *Aedes aegypti*. *Spilanthus acmella* flower extract showed more effect than the *Andrographis paniculata*. These plants which have insecticidal properties are seemed to be better vector control agent than the synthetic pesticides.

INTRODUCTION

Mosquito have much greater influence on human health and well-being throughout the world than any other arthropod, mainly because of their involvement in both transmitting a number of dreadful diseases such as malaria, filarial, dengue, chikungunya etc and create nuisance to public health. These medically important mosquitoes which act as a vector for the transmission of serious diseases that causes morbidity, mortality, economic loss and social disruption¹.

Mosquitoes were recognized as health nuisance problem only in the last century. Among the various control measures used, the biological control becomes a necessity. Although vector control programs have been established for a long time, the main method for control of vector is the use of chemical insecticides². However, the effectiveness of vector control has declined due to the development of resistance in mosquitoes³. An alternative approach for mosquito control is the use of natural products of plant origin. The botanical insecticides are generally pest specific, readily bio-degradable and usually lack toxicity to higher animals⁴.

The first extensively used compound pyrethrum against mosquito was obtained from the flower of *Chrysanthemum cinerariaefidum*. Although the extract is effective yet it is photogenerative and requires high quantity to control the insect population. Hence, the investigations for such phyto-compounds are renewed which have selectively higher toxicity at low concentration to various or all developmental stages of mosquito⁵.

Many research work were carried out to know the efficacy of natural products as larvicides^{6,7}. Ethanolic extract of *Spilanthes acmella murr.* have more active compound than DDT against *Anopheles larva*⁸.

Spilanthes acmella (L) Murray is also known as Toothache plant and its various synonyms are *Bidenes acmella*, *Pyrethrum acmella*, *Verbesina acmella* etc. The plant belongs to family compositae, an annual herb up to 30 – 60 cm in height give burning taste as a remedy for stammering and toothache. Other aerial parts and roots are used for curing of inflammation and diarrhea. The flower tops and aerial parts have been found to be toxic to mosquito larvae and *Periplanata*. The compound spilanthol has been identified as having larvicidal activity⁹.

Andrographis paniculata commonly known as “King of Bitter” or Rice bitter¹⁰. *Andrographis paniculata* is an annual branch, erect running ½ to 1 meter in height. It is a member of the plant family Acanthaceae, and grows abundantly in South eastern Asia, India, Srilanka, Pakistan, Indonesia, China etc. It has been used for certain in Asia to treat Gastrointestinal (GI) tract infections, respiratory infections, malarial fever¹¹, herpes and as antibacterial, vermifugal, filaricidal besides as an insect repellent and in a variety of chronic and infectious diseases¹². The present study deals with the laboratory investigations to ascertain the larvicidal properties of *S. acmella* and *A. paniculata* on the larvae of mosquito species *A. aegypti* a vector for chikungunya and dengue.

MATERIALS AND METHODS

Collection of plants and extraction

Fresh plant materials of each of the selected species were collected at the peak of the dry season in January 2008. Specimens of all the plant species studied were deposited in herbaria of our institute. The selected plant materials were powdered by an electrical blender. From each sample, 100g of plant material was extracted with 300ml of appropriate solvent for 8hrs in a soxhlet apparatus¹³. The extract liquid was subjected to rotary evaporation in order to keep in refrigerator for their use. The dried residues were weighed and dissolved in acetonitrile solvent to prepare the stock solution.

Collection of test animals

The eggs of *Aedes aegypti* were collected from National Institute of Communicable Disease Centre- Coimbatore. The larvae were cultured and maintained in the laboratory at $27 \pm 1^{\circ}$ and 85% relative humidity. Larval forms were maintained in trays by providing dog biscuit and yeast powder in the 3:1 ratio. Adult mosquitoes were maintained in a net cage (90X90X90 cm) and were continuously supplied 10% sucrose solution with a cotton wick. For continuous culture selected number of mosquitoes were allowed to feed chicken blood every third day, thereafter moist filter paper was kept in beaker in the cage for mosquitoes to lay their eggs. Eggs laid on the filter paper were immersed in larval basins containing water for the maintenance of the colony.

Test for larvicidal activity

Mosquito larvicidal assays were carried out according to WHO¹⁴ standard procedures, with slight modification. For experimental treatment the extract was diluted in

absolute ethanol and added 99 ml of distilled water in 150ml disposable wax coated paper cup, which was shaken lightly to ensure a homogeneous test solution. Then 25 early fourth instar larvae of vector mosquitoes were transferred to each cup. Each experiment was performed in 4 replicates with a final total of 100 larvae for each concentration and equal number of control was setup simultaneously with 1ml of ethanol mixed with 99ml of distilled water. The mortality was determined after 24h of exposure, during which time no food was offered to the larvae. The control mortality was corrected by Abbott's formula¹⁵. And Lc50 and Lc90 regression and 95% confidence limit was calculated by using probit analysis¹⁶.

RESULTS AND DISCUSSION

The results of the larval and pupal susceptibility of *A. aegypti* using the 2 plant extract are presented in the Table: 1. the acetic extract of both plant materials were effective against larvae and pupae of mosquito. The effect of larval mortality was concentration dependent. The Lc50 and Lc90 values of both plant extracts against II and IVth instar larvae and pupae are presented in the Table 2. The Lc50 and Lc90 of II instar of *Spilanthes* plants were 22.97, 226.27ppm; 56.23, 221.19ppm for IV instar and 154.26, 221.19ppm for pupae respectively. In the *Andrographis* leaf extract II instar larvae Lc50 and Lc90 were 48.24, 223.15ppm; 122.35, 278.61ppm in IVth instar and 210.53, 352.05ppm in pupae respectively.

Table 1: Larval and pupal mortality percentage of *Aedes aegypti* for different concentrations of *Spilanthes acmella* flower extract and *Andrographis paniculata* for 24 hr exposure

Plants used	Stages of exposure	Experimental concentration in ppm						
		Control	10	50	100	150	200	300
<i>Spilanthes acmella</i>	II instar Larval Mortality %	0	36.67	63.33	78.33	90.00	91.67	90.00
	IV instar Larval Mortality %	0	25.00	51.67	66.67	85.00	91.67	100.00
	Pupae Mortality %	0	15.00	16.67	36.67	56.67	70.00	75.00
<i>Andrographis paniculata</i>	II instar Larval Mortality %	0	35.00	56.67	68.33	78.33	80.00	91.67
	IV instar Larval Mortality %	0	23.33	35.00	43.33	60.00	75.00	91.67
	Pupae Mortality %	0	1.67	6.67	13.33	35.00	45.00	78.33

Table 2: Showing the Lc50 and Lc90 values of *Spilanthus acmella* flower extract and *Andrographis paniculata* against the different stages of mosquito *Aedes aegypti* under 24hr exposure

Plants used	Stages of exposure	LC 50 (ppm)	Lc 90 (ppm)	Regression
<i>Spilanthus acmella</i>	II instar	22.97	226.27	0.6833
	IV Instar	56.23	221.19	0.8716
	Pupae	154.26	221.19	0.9103
<i>Andrographis paniculata</i>	II instar	48.24	223.15	0.8845
	IV Instar	122.35	278.61	0.987
	Pupae	210.53	352.05	0.9755

Flower extract of *Spilanthus* plant was found to possess the most effective larvicidal activity against *Aedes aegypti*. The insecticidal potency of the whole plant acetonc extract of *Andrographis paniculata* against both larvae and pupal mosquitoes was lower compared to *Spilanthus acemella Mur*. Exposure time also has crucial role in causing toxicity. It is observed that as the concentration and exposure time increases, progressively mortality also increases in severity.

Plant alkaloids resulted in a significant loss in fecundity and fertility in the adult species of mosquitoes¹⁷. Leaf extract of *Vitex negundo*, *Nerium oleander* and seed of extract of *Syzygium jambolanum* at very low concentration showed larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi* and also extended the duration of larval instar population¹⁸. Pericarp of *Zanthoxylum limonella* was found to have the most promising larvicidal properties against *Aedes albopictus* and *Culex quinquefasciatus*.

Behavioral changes like abnormal spiral movement, incapability to reach 3 – 4 inch high from the bottom of water column, strong jerky movements exhibited intermittently by experimental larvae were also noticed which may be due to toxicant stress. *Spillanthol* is more effective even at low doses against the larvae and pupae and affects the nerve conduction. Similar observation also noted in our study. *Spilanthus* greatly disturbed the ongoing process of histolysis and histogenesis⁹. Thus our study conform the larvicidal property of the plant. The isolation of the active compound form the plant will help to control mosquito population in the near future.

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